Application Note

PHYLUCE is a software package for the analysis of conserved genomic loci

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Abstract

Targeted enrichment of conserved and ultraconserved genomic elements allows universal collection of phylogenomic data from thousands of species. Prior to downstream inference, data from these types of targeted enrichment studies must undergo pre-processing to assemble contigs from sequence data; identify targeted, enriched loci from the off-target background data; align enriched contigs representing conserved loci to one another; and prepare and manipulate these alignments for subsequent phylogenomic inference. PHYLUCE is an efficient and easy-to-install software package that accomplishes these tasks across hundreds of taxa and thousands of enriched loci.

Availability and Implementation

PHYLUCE is written for Python 2.7. PHYLUCE is supported on OSX and Linux (RedHat/CentOS) operating systems. PHYLUCE source code is distributed under a BSD-style license from https://www.github.com/faircloth-lab/phyluce/. PHYLUCE is also available as a package (https://binstar.org/faircloth-lab/phyluce) for the Anaconda Python distribution that installs all dependencies, and users can request a PHYLUCE instance on iPlant Atmosphere (tag: phyluce-1.5). The software manual and a tutorial are available from http://phyluce.readthedocs.org/en/latest/ and test data are available from doi: 10.6084/m9.figshare.1284521.

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Supplementary information: None

Target enrichment of conserved and ultraconserved elements (hereafter "conserved loci") allows universal phylogenomic analyses of non-model organisms at multiple time scales (Faircloth et al. 2012; Faircloth et al. 2013; Smith et al. 2014; Faircloth et al. 2015). The strength of the approach derives from its ability to universally collect sequence data from thousands of loci across thousands of species, permitting phylogenetic comparisons across Classes and higher taxonomic ranks. When the goal of data collection is to infer the evolutionary history of species, the subsequent analytical tasks are generally to: (1) assemble the sequencing reads, which may span tens to hundreds of individuals; (2) identify putative orthologs among the assembled contigs on a sample-by-sample basis while removing putative paralogs; (3) allow the flexible creation of datasets containing different individuals, individuals included from other experiments, or individual genome sequences; (4) identify and export sequence data from orthologs across all individuals in the set; (5) align the data and optionally trim resulting alignments in preparation for phylogenetic inference; (6) compute summary statistics on the aligned data; and (7) perform utility functions on the sequence or alignment data to prepare them downstream analyses using a variety of phylogenetic inference programs. *PHYLUCE* (pronounced "phy-loo-chee") is the first open-source, easy-to-install software package to perform these tasks in a computationally efficient manner.

The *PHYLUCE* workflow (Fig 1) for inferring phylogeny begins with external preparation of sequence reads from target-enriched libraries by trimming adapter contamination and low-quality bases using a program like Trimmomatic (Bolger et al. 2014) or a batch processing script similar to *illumiprocessor* (Faircloth 2013). *PHYLUCE* then offers several programs to batch-assemble the resulting "clean" reads into contigs using different assembly programs (Zerbino and Birney 2008; Simpson et al. 2009; Grabherr et al. 2011) with parallelization approaches tailored to each program. The next step in the PHYLUCE workflow is to identify orthologous conserved loci shared among individuals. The MATCH_CONTIGS_TO_PROBES program performs the steps of ortholog identification and paralog removal by aligning the assembled contigs to a FASTA file of target enrichment baits using *lastz* (Harris 2007). Although this program is designed to work with standardized baits sets developed for the targeted enrichment of UCE loci (e.g. http://ultraconserved.org), users can input custom bait sets with different naming conventions (Mandel et al. 2014) by adjusting several parameters. Following the alignment step, MATCH_CONTIGS_TO_PROBES screens the *lastz* output to identify (1) assembled contigs hit by probes targeting different loci, and (2) different contigs that are hit by probes targeting the same locus. The program assumes that these reciprocally duplicate loci are potentially paralagous and removes them from downstream analytical steps. The program then builds a

relational database containing a table of detections and non-detections at each locus across all input assemblies as well as a table associating the name of each targeted locus (from the FASTA file representing the bait set) with the name of the assembled contig to which it matches. Next, users of PHYLUCE create a "taxon-set" configuration file that specifies the individual assemblies that will be used in downstream phylogenetic analyses. By inputting this configuration file to the GET_MATCH_COUNTS program, users can flexibly create different data sets, integrate data from separate studies targeting the same loci, or include identical loci harvested from extant genome sequences (e.g. http://github.com/faircloth-lab/uce-probe-sets). After identifying those individuals and loci in the desired taxon set, users extract the contigs corresponding to non-duplicate conserved loci into a monolithic (all loci for all taxa) FASTA-formatted file using the GET_FASTAS_FROM_MATCH_COUNTS program. This program renames each contig for each species within the taxon set such that the FASTA header for each contig contains information denoting the species in which the conserved locus was detected and the specific conserved locus to which it matched. After creating the monolithic FASTA, users can align the targeted loci with the SEQCAP_ALIGN program, which parallelizes MAFFT (Katoh and Standley 2013) or MUSCLE (Edgar 2004) alignments across all targeted loci on computers with multiple CPUs. The SEQCAP_ALIGN program also offers the option to trim the resulting alignments for edges that are poorly aligned - a suitable choice when the species within the taxon set are closely related (e.g., Order-level or lower taxonomic ranks). For more conservative alignment trimming, PHYLUCE offers GET_GBLOCKS_TRIMMED_ALIGNMENTS_FROM_UNTRIMMED which is a program that implements parallelized, internal trimming using Gblocks (Castresana 2000; Talavera and Castresana 2007). PHYLUCE includes several parallelized programs to manipulate the resulting alignments, including the ability to rapidly generate summary statistics across thousands of alignments, explode alignments into their corresponding FASTA sequences, extract taxa from alignments, compute parsimony informative sites within alignments, and convert alignments between common formats. After alignment, PHYLUCE users can generate data matrices having varying levels of completeness using the GET_ONLY_LOCI_WITH_MIN_TAXA program. This program screens each locus for taxonomic completeness and filters out those loci containing fewer taxa than desired. In this way, users can create 100% complete (all taxa have data for all loci) or incomplete data matrices (some loci have data for a certain percentage of taxa). After filtering loci for taxonomic completeness, PHYLUCE offers several programs to format the resulting alignments for analyses in PartitionFinder (Lanfear et al. 2012), RAxML (Stamatakis 2014), ExaML (Kozlov et al. 2015), ExaBayes (Aberer et al. 2014), GARLI (Zwickl 2006), or

MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Programs are also available to assist users with preparing data for and running gene-tree-based species tree analyses.

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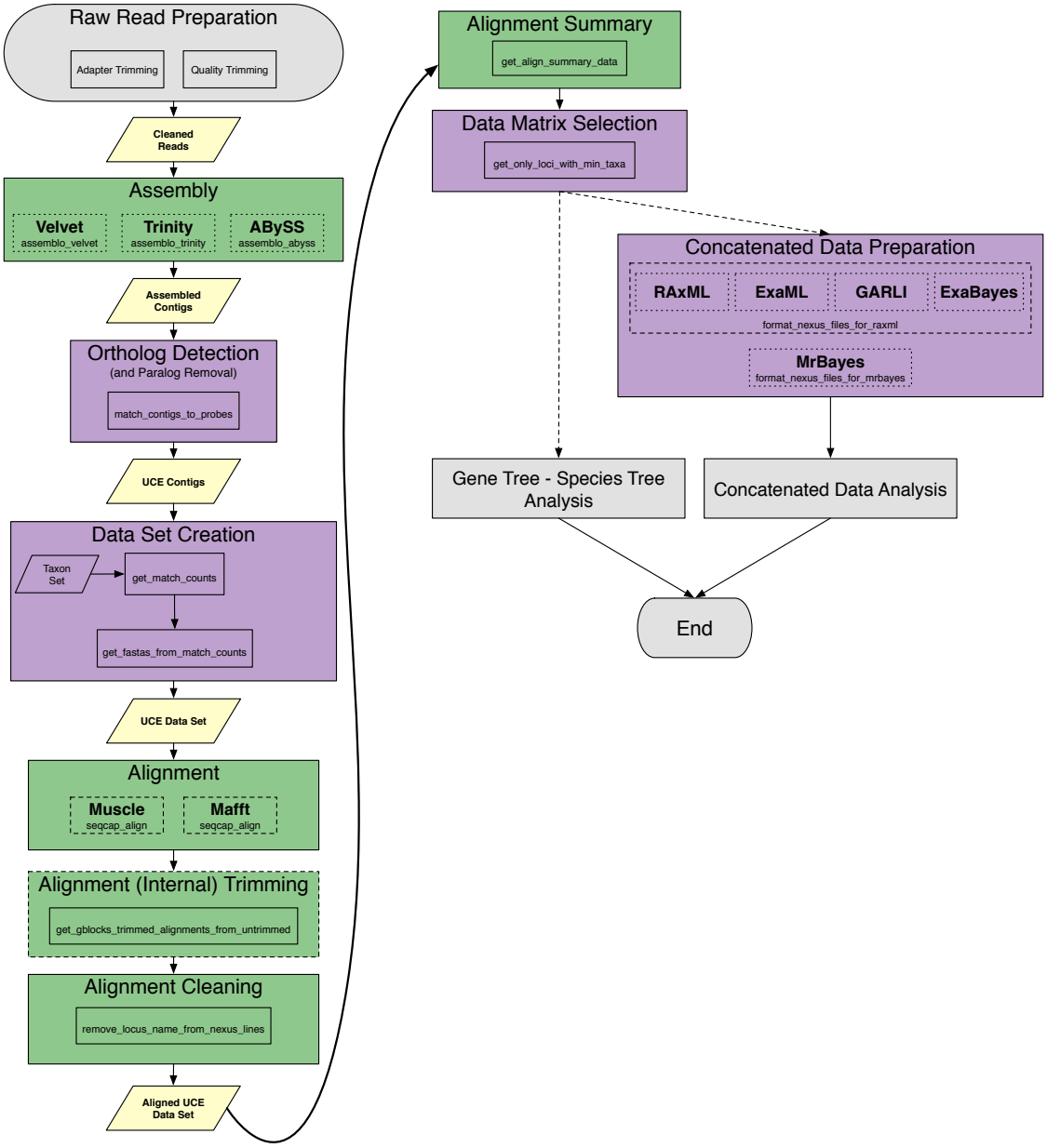
Conflict of Interest

None

Figure 1. PHYLUCE workflow for phylogenomic analyses of data collected from conserved genomic loci using targeted enrichment.



Aberer,A.J. *et al.* (2014) ExaBayes: massively parallel bayesian tree inference for the whole-genome era. *Mol. Biol. Evol.*, **31**, 2553–2556.

Bolger,A.M. *et al.* (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114–2120.

Castresana,J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.*, **17**, 540–552.

Edgar,R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, **5**, 113–119.

Faircloth,B.C. *et al.* (2013) A phylogenomic perspective on the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved elements (UCEs). *PLoS One*, **8**, e65923.

Faircloth,B.C. (2013) illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming.

Faircloth,B.C. *et al.* (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Mol. Ecol. Resour.*, **15**, 489–501.

Faircloth,B.C. *et al.* (2012) Ultraconserved Elements Anchor Thousands of Genetic Markers Spanning Multiple Evolutionary Timescales. *Syst. Biol.*, **61**, 717–726.

Grabherr,M.G. *et al.* (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.*, **29**, 644–U130.

Harris,R.S. (2007) Improved pairwise alignment of genomic DNA.

Huelsenbeck,J.P. and Ronquist,F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Katoh,K. and Standley,D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.*, **30**, 772–780.

Kozlov,A.M. *et al.* (2015) ExaML version 3: a tool for phylogenomic analyses on supercomputers. *Bioinformatics*, **31**, 2577–2579.

Lanfear,R. *et al.* (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.*, **29**, 1695–1701.

Mandel,J.R. *et al.* (2014) A target enrichment method for gathering phylogenetic information from hundreds of loci: An example from the Compositae. *Applications in Plant Sciences*, **2**.

Ronquist,F. and Huelsenbeck,J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Simpson,J. *et al.* (2009) ABySS: a parallel assembler for short read sequence data. *Genome Res.*, **19**, 1117–1123.

Smith,B.T. *et al.* (2014) Target Capture and Massively Parallel Sequencing of Ultraconserved Elements (UCEs) for Comparative Studies at Shallow Evolutionary Time Scales. *Syst. Biol.*, **63**, 83–95.

Stamatakis,A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.

Talavera,G. and Castresana,J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.*, **56**, 564–577.

Zerbino,D.R. and Birney,E. (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.*, **18**, 821–829.

Zwickl,D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.